No. II.

November 5.—Gerbil (F. 11) injected with blood (0·2 c.c.) obtained as above, but after two hours' standing no trypanosome could be seen, only round forms and free granules.
November 12.—Trypanosomes first found in blood.
November 14.—Trypanosomes very numerous.
November 15.—Gerbil found dead; spleen very large.

The average time of infection in gerbils is four to six days after ordinary inoculation. Similar results were also obtained with dogs.

These experiments are, of course, not absolutely conclusive, but so far as could be ascertained microscopically the granules were the only discernible remnants of the trypanosomes which retained their characteristic form.

Further experiments were also made to trace if possible the fate of granules so injected into animals. Inoculations were made with solutions containing large number of free granules, and the animals were killed before trypanosomes could be found in the blood. Granules and the later forms in various stages of development were found in the proximal glands, also in the internal organs.

Note on a New Method of Blood Fixation.

By H. G. Plimmer, F.R.S.

During some years of work on the blood of animals, many methods of fixation have been tried, principally with the view of obtaining a better fixation of blood parasites. The method described below has fulfilled this object better than any other, and is more faithful than even osmic acid.

The use of iodine for the fixation of unicellular organisms dates from the work of Kent in 1881 on the Infusoria, but the application of it to blood is, so far as I know, new.

I have used iodine in two forms, in vapour and in solution, and each has its special advantages. When a blood-film is exposed wet to the vapour from a solution of iodine in chloroform, the fixation of the various elements is practically instantaneous, as the penetrative power of iodine in this form is greater than that of any other fixative known to me; there is less alteration both in form and size of the cellular elements and parasites than with any other fixative. When used in solution several things happen which are of value in enabling very fine structures to be more easily made out.

If blood be mixed with a solution of iodine in salt solution containing iodide of potassium, certain elements and parasites, especially trypanosomes, swell up so that the finer parts of their structure, for instance the nucleus and blepharoplast, are much clearer and more definite than with the ordinary

methods. The nucleus shows as clearly as, if not clearer than, when Flemming's solution and iron-hæmatoxylin have been used. There is the clear space containing the karyosome, and surrounding this, in many cases, are seen a number of granules, some of which can be seen budding off. The blepharoplast is clearly seen as a structure quite distinct from the micronucleus, and the earlier stages of division of a trypanosome, *i.e.* the division of the blepharoplast and the formation of a second undulating membrane extending down the body of the trypanosome and forming eventually a second flagellum, can be seen and followed easier than with any other mode of fixation. For the smaller forms found in spleen, glands, and marrow of animals with chronic trypanosomiasis, this method, by causing swelling of the elements, renders the very small forms distinct, and renders their nuclear structures much more visible.

Both these methods are also the best I have found for avian and reptilian blood containing parasites, *e.g.* filaria, malaria, hæmogregarines, etc.

The steps of the two methods are here detailed. Either slides or cover-glasses can be used, but in all blood-work the best results are obtained with cover-glasses. After the Giemsa or fuchsin staining the definition is greatly increased by the use of a green monochromatic screen, such as Wratten's No. 19, which shows the picture in blacks and greys.

I. *Vapour Method.*

1. Expose the thinnest possible film whilst wet to the vapour of a solution of iodine in chloroform for 10–15 seconds until it is distinctly yellowish.

A hollowed glass block does for cover-glasses, and a glass cylinder of suitable height, with the iodine and chloroform in a small vessel at the bottom, does for the slides. In cold places the vessel should be warmed in order to get the vapour given off freely.

2. Place the film when it has become just surface dry (a dead, mat surface, not really dry) in chloroform, or in alcohol and ether, equal parts, for two hours. I use chloroform for cover-glasses and alcohol-ether for the rougher slides.

3. There will now be no free iodine left in the film, and it can be stained in many ways. I use the following :—

A. *a.* Drop 3–8 drops of Giemsa's solution on the film, and immediately after double the number of drops of distilled water. Leave for from 2 to 12 hours.
   b. Wash well with tap-water.
   c. Drop on 2–8 drops of orange-tannin solution and leave for 15 seconds.
   d. Wash thoroughly with tap-water, up to two minutes.
   e. Dry with filter-paper.
   f. Mount in cedar oil or liquid paraffin.

B. *a.* Carbol-fuchsin for from 2 to 12 hours.
   b. Wash in tap-water.
   c. Alcohol until free from bulk of stain.
   d. Differentiate in clove oil saturated with orange G.
   e. Stop when desired by washing in xylol.
   f. Mount in cedar oil or liquid paraffin.

C. Iron-hæmatoxylin may be used in any of the ordinary ways. Kernschwarz for 24 hours gives very delicate results.

II. *Solution Method.*

1. Make a saturated solution of potassium iodide in 0·8-per-cent. salt solution and add iodine to saturation.

2. Mix 5–6 drops of this with 10 c.c. of salt solution.

3. Mix in a marked pipette equal parts of this and the blood to be examined. In the case of organs small pieces may be crushed in an equivalent quantity of the iodine solution to form an emulsion.

4. Take large drops and make a thickish film. Wait until the surface has begun to dry (as in I), and place in alcohol and ether for two hours.

5. Continue as under 3.

DESCRIPTION OF PLATES.

Plate 9.

Fig. 1.—Series to illustrate mechanism of extrusion of granules in *T. nanum* (see p. 380).

„ 2.—Developmental forms of *T. nanum*, seen in bone-marrow; the progressive tendency towards the characteristic shape of the adult trypanosome is shown. Dark-ground illumination, Leitz $\frac{1}{12}$ objective, N.A. 1.30, compensating eye-piece. ×8.

The earliest form, A, shows no evidence of a protoplasmic envelope and has the appearance of a well-developed granule just after extrusion. In B the cytoplasm is clearly evident and the separation of the micronucleus has commenced. C shows a well-developed form, of circular shape, with the nuclei shown at a distance from each other.

D, E, F, and G show the progressive increase of protoplasm, the last form being almost trypanosomal. H is a young trypanosome, and I an older one in which a flagellum is evident.

These forms were all living when drawn.

Plates 10 and 11.

All the figures are drawn under a Zeiss 3-mm. apochromatic objective, N.A. 1.40, with compensating ocular. ×12.

Plate 10.

Figs. 1–8.—*T. rhodesiense* in rat's blood, showing granules from their origin to extrusion.

Figs. 9–16.—From blood and liver of rat infected with *T. rhodesiense*.

Figs. 17–22, 24, and 26.—Are from the spleen of a guinea-pig infected with Nagana which lived three months, and showed no trypanosomes in the blood for some time before death.

Figs. 23 and 25.—From a lymphatic gland of a cat infected with Nagana.

Fig. 1.—Four granules are seen in the trypanosome-body, and another is in an early stage of being budded off from the macronucleus at the right upper angle.

„ 2.—Two granules are seen coming off the macronucleus. The one on the left is still attached and shows the elongated form.

Fig. 3.—A similar elongated granule is seen completely separated from the nucleus. There is a faint indication of a halo surrounding it.

„ 4.—A large elongated granule is seen between the macro- and micronuclei, lying close to the periplast.

„ 5.—Several granules are present; one is just being detached from the macronucleus.

„ 6.—Two granules are seen on the point of escaping from the trypanosome; the larger looks as if it is nearly extruded.

„ 7.—A recently extruded granule is seen near the trypanosome. The macronucleus shows two deeply stained points—probably granules becoming differentiated in its substance before being budded off.

„ 8.—Two granules, lying between the macro- and micronuclei, are each seen to be surrounded by a well-defined clear hyaline area. Two others are almost completely separated from the macronucleus.

„ 9.—Free granule; no differentiation.

„ 10.—Free granule, larger, and with a faint rim of cytoplasm.

„ 11.—Ring-shaped nucleus with micronucleus coming off; definitely more protoplasm than the previous form.

„ 12.—Early form with macro- and micronucleus and pale blue-staining cytoplasm.

„ 13.—Similar form, larger.

„ 14.—The nucleus has divided in this specimen, while there is only one micronucleus seen.

„ 15.—Both macro- and micronuclei are divided.

„ 16.—Micronuclei only have divided; macronucleus in process of division.

„ 17–26.—All are similar forms. They vary in shape and correspond closely with the forms seen by vital staining of emulsions of internal organs.

„ 20 and 22.—Show division of the micronuclei.

„ 21 and 22.—Show the third chromatin body described.

„ 25.—Shows division of macro- and micronuclei.

„ 27.—A single form, with macro- and micronucleus, and a very long flagellum.

Plate 11.

Figs. 1–12.—The specimens were found in smear preparations from the liver and kidney from rats infected with *T. rhodesiense*. They show dividing forms in various stages.

Figs. 13–20.—Blood from liver of rat infected with *T. rhodesiense*. Immature trypanosomes are shown gradually merging into adult forms.

Fig. 1.—Early stage of division. There are already two micronuclei, but the macronucleus is just beginning to divide.

„ 2.—This shows similar division to fig. 1, but a little further advanced. The macronucleus is now in the stage of mitosis.

„ 3.—Complete separation of macro- and micronuclei, but the flagella are not yet separated.

„ 4.—Two form with nuclei and flagella completely divided; one flagellum is much longer than the other and lies round the margin of the body.

„ 5.—Two form beginning to divide into two independent bodies which are identical with the early immature forms shown in figs. 13-15.

„ 6.—Two form. The nuclei have moved to some distance from each other. A thick fan is seen in the shorter of the two flagella.

Fig. 1.

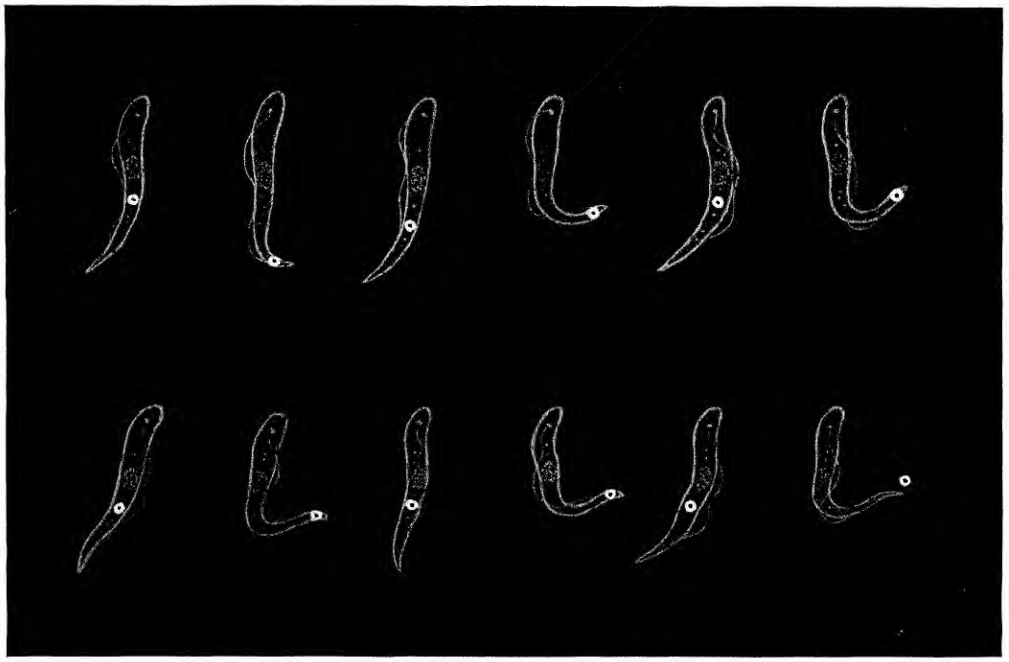
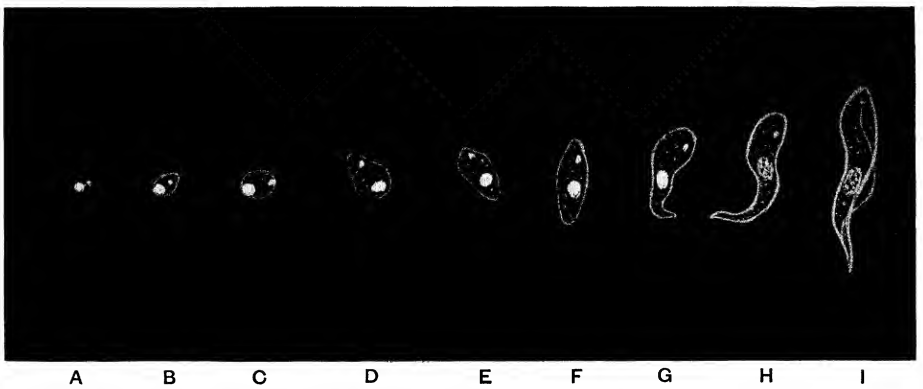


Fig. 2.

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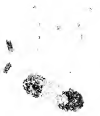

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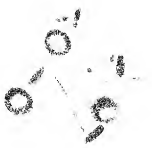

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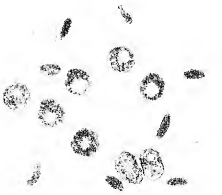

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Fig. 7.—Four form (early), the macronuclei have evidently recently divided. The two lower are moving away from each other; the upper have not completely separated.

„ 8.—More advanced four form; all the pairs of macro- and micronuclei have moved away from each other.

„ 9.—Eight form, a large body of cytoplasm whose margin shows a few indentations as if there might later be division of the whole mass at these situations. All the macro- and micronuclei and flagella can be seen.

„ 10.—Eight form beginning apparently to divide; the cytoplasm shows lines of cleavage along the lower part of the outline.

„ 11.—Mass of 16 bodies breaking up. These resemble the Leishman-Donovan body; each has a macro- and micronucleus, but no flagellum.

„ 12.—A large form with single macronucleus and large micronucleus showing fan-shaped origin to flagellum.

„ 13.—The body is rounded and has a clear blue-staining cytoplasm. The flagellum shows the fan-shaped origin well and stands straight out from the body. The micronucleus lies close to the macronucleus.

„ 14 and 15.—The body is longer, and the flagellum is lying along the margin; the micronucleus is now moving away from the macronucleus.

„ 16, 17, and 18.—These features are more marked, and the specimens show gradual approximation to adult type. The flagellum is seen to be separated at some point from the outline of the trypanosome body, the earliest stage in the development of an undulating membrane.

„ 19.—The undulating membrane is now clearly present, but the trypanosome can still be recognised as immature by the fan-shaped origin of the flagellum and the pale homogeneous cytoplasm.

„ 20.—An early adult trypanosome; the flagellum no longer shows the fan-shaped origin, and is much longer. Early granules can be seen in the cytoplasm.

Fig. 1.

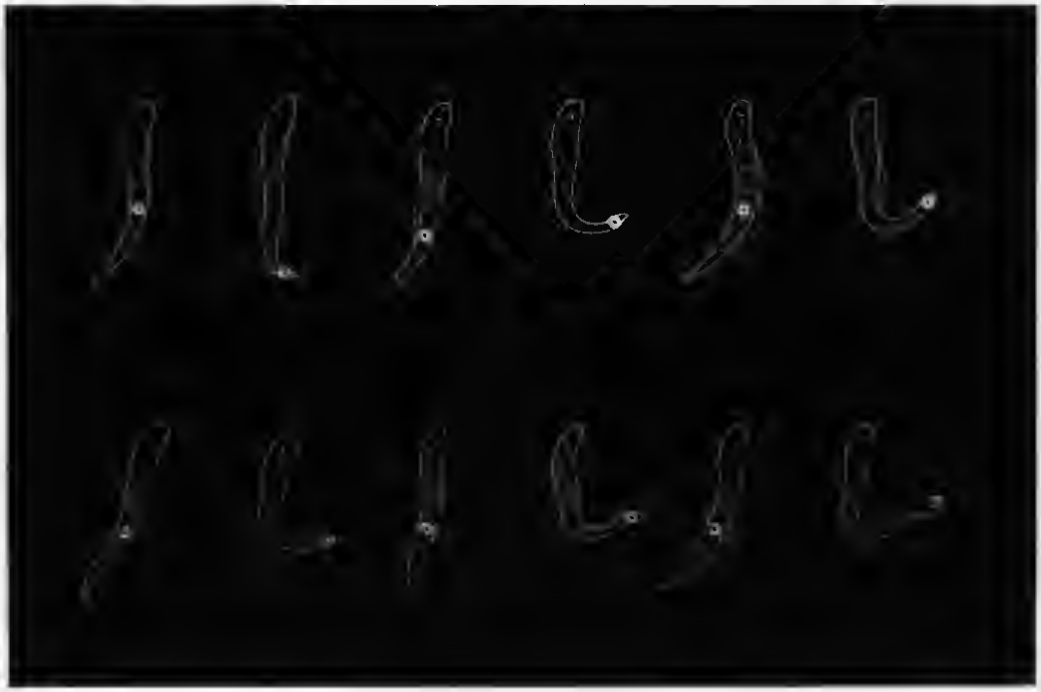
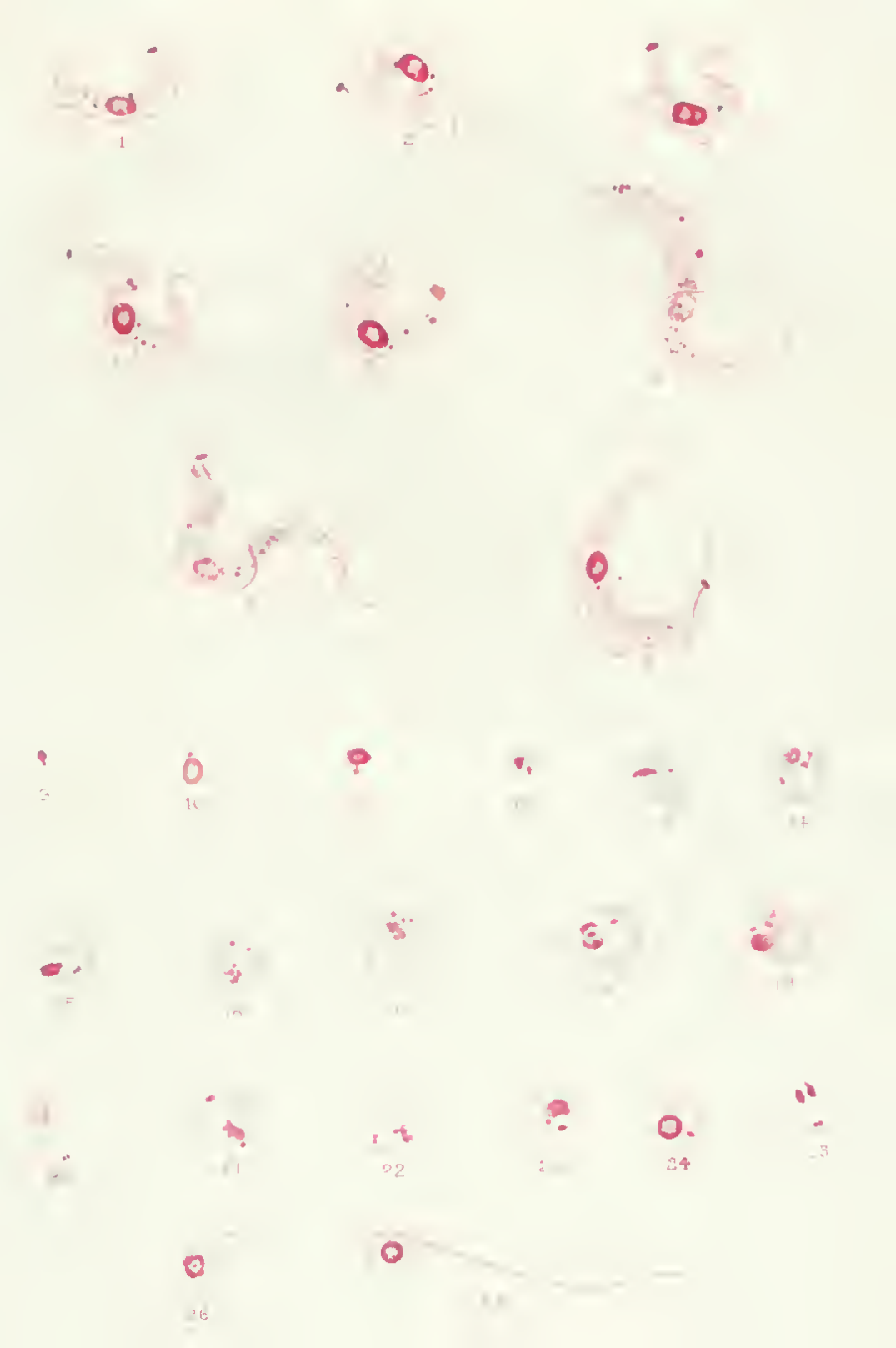


Fig. 2.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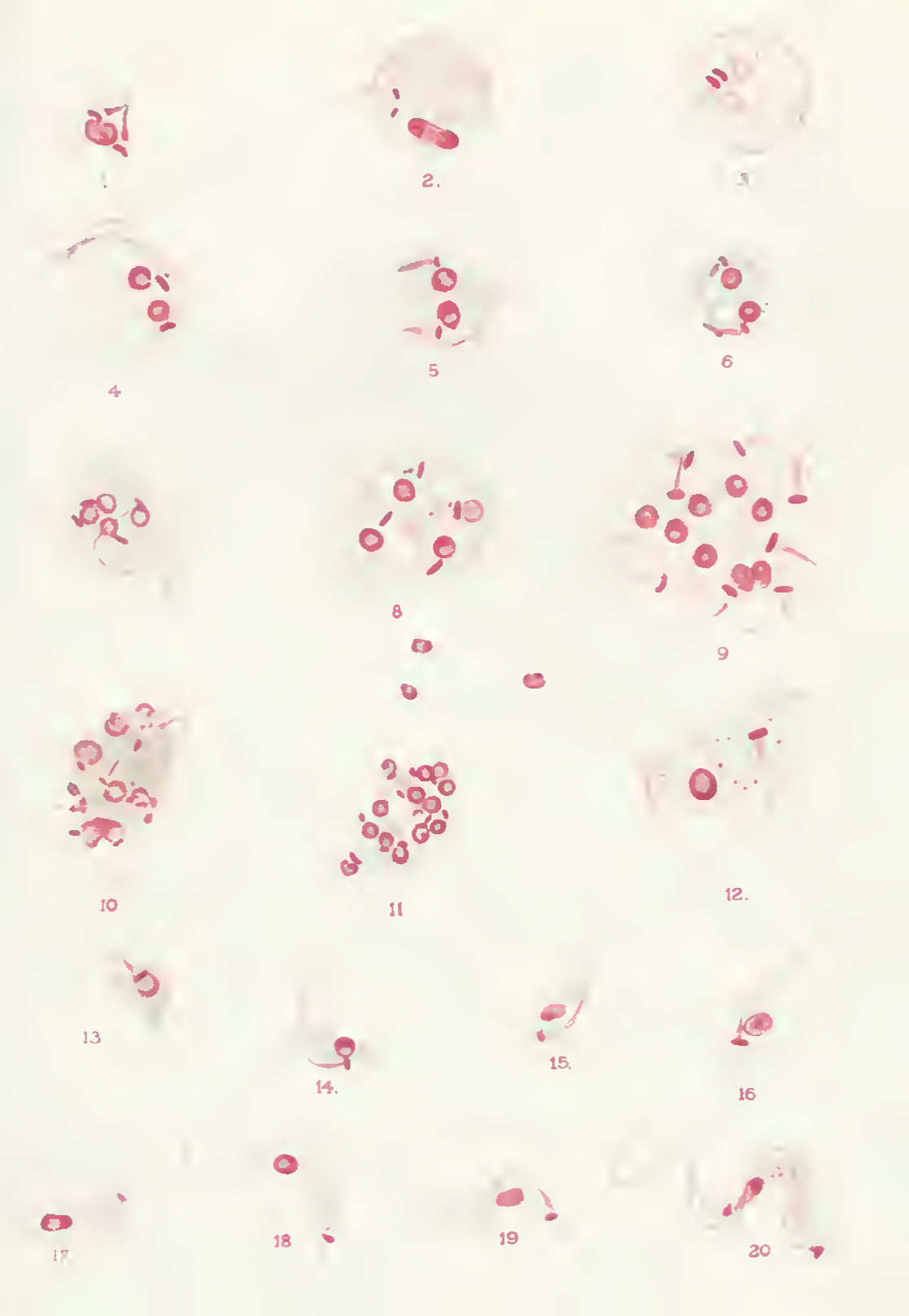
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